Applicants: Romero et al. Serial No.: 10/511,384 Filed: October 15, 2004

Response to Office Action Dated March 12, 2008

Page 2 of 15

IN THE SPECIFICATION:

Please insert the attached substitute sequence listing after the specification but before the claims.

On pages 18 and 19 of the specification, please replace Tables 1 and 2 with the following new tables:

Table 1. Estimation of the VEGF protein family MHCI associated peptides in the context of HLAA.0201

| | | | | | | A1 | A Using BIMAS software | ware | | | | | | |
|----------|-----------|-------|--------|-----------|-------|--------|----------------------------|---------|--------|-----------|-------|--------|-----------|-------|
| | VEGF-A | | | VEGF-B | | | VEGF-C | | | VEGF-D | | | PIGF | |
| SEQ | Secuencia | Kd | SEQ ID | Secuencia | β | SEQ ID | Secuencia | Kd | SEQ ID | Secuencia | Kd | SEQ ID | Secuencia | ğ |
| 27 | LLSWVHWSL | 272 | 37 | LLLAALLOL | 309 | 47 | YLSKTLFEI | 640 | 57 | FMMLYVQLV | 1966 | 67 | RLFPCFLQL | 150 |
| 28 | ALLLYLHHA | 42 | 38 | QLAPAQAPV | 20 | 48 | TLFEITVPL | 324 | 28 | KLWRCRLRL | 620 | 89 | VVSEYPSEV | 42 |
| 29 | WSLALLLYL | 30 | 39 | QLVPSCVTV | 20 | 49 | VLYPEYWKM | 304 | 59 | QLFEISVPL | 324 | 69 | VMRLFPCFL | 42 |
| 30 | FLQHNKCEC | 23 | 40 | LMGTVAKQL | 26 | 20 | CMNTSTSYL | 85 | 09 | YISKQLFEI | 88 | 70 | RALERLVDV | 34 |
| 31 | WYHWSLALL | 20 | 41 | LLAALLQLA | 19 | 51 | KLFPSQCGA | 64 | 61 | CMNTSTSYI | 41 | 7.1 | VELTFSQHV | 32 |
| 32 | FLLSWVHWS | 16 | 42 | LLQLAPAQA | 8 | 52 | LLGFFSVAC | 32 | 62 | VLQEENPLA | 35 | 72 | AVPPQQWAL | 14 |
| 33 | RQLELNERT | 9 | 43 | VVSWIDVYT | 9 | 53 | SLPATLPQC | 11 | 63 | WVVVNVFMM | 27 | 73 | LQLLAGLAL | 4 |
| 34 | NITMQIMRI | 3 | 44 | суртванау | 9 | 54 | GLQCMNTST | 7 | 64 | VNVFMMLYV | 10 | 74 | RSGDRPSYV | 10 |
| 35 | YCHPIETLV | 2 | 45 | KQLVPSCVT | 4 | 99 | AAFESGLDL | 4 | 99 | SLICMNTST | 7 | 75 | LLAGLALPA | 80 |
| 36 | IEYIFKPSC | 2 | 46 | VVVPLTVEL | 3 | 56 | EQLRSVSSV | 4 | 99 | CVLQEENPL | 7 | 76 | CVPVETANV | 9 |
| | | | | | | B Us | B Using SYFPEITHI software | oftware | | | | | | |
| | VEGF-A | | | VEGF-B | | | VEGF-C | | | VEGF-D | | | PIGF | |
| SEQ O | Secuencia | Score | SEQ ID | Secuencia | Score | SEQ ID | Secuencia | Score | SEQ ID | Secuencia | Score | SEQ ID | Secuencia | Score |
| 22 | TEMAHMST | 24 | 87 | LLLAALLQL | 29 | - 26 | TLFEITVPL | 27 | 107 | FMMLYVQLV | 25 | 117 | ALERLYDVV | 26 |
| 78 | ALLLYLHHA | 24 | 88 | QLAPAQAPV | 26 | 86 | DLEEQLRSV | 26 | 108 | QLFEISVPL | 25 | 118 | RLFPCFLQL | 24 |
| 79 | WYHWSLALL | 20 | 89 | QLVPSCVTV | 26 | 66 | YLSKTLFEI | 26 | 109 | YISKOLFEI | 24 | 119 | RALERLVDV | 24 |
| 80 | SLALLYLH | 20 | 96 | VVVPLTVEL | 54 | 100 | ALLPGPREA | 24 | 110 | KLWRCRLRL | 23 | 120 | LLAGLALPA | 22 |
| 81 | SYCHPIETL | 19 | 91 | LLRRLLLAA | 23 | 101 | CMNTSTSYL | 21 | 111 | RAASSLEEL | 22 | 121 | LAGLALPAV | 22 |
| 82 | NITMQIMRI | 19 | 95 | LLAALLQLA | 23 | 102 | DICGPNKEL | 21 | 112 | SLEELLRIT | 22 | 122 | VMRLFPCFL | 20 |
| 83 | FLLSWVHWS | 18 | 93 | FLRCQGRGL | 22 | 103 | AAAAFESGL | 20 | 113 | ATFYDIETL | 22 | 123 | CFLQLLAGL | 20 |
| 84 | WSLALLLYL | 18 | 94 | LTVELMGTV | 74 | 104 | AAFESGLDL | 20 | 114 | EISVPLTSV | 22 | 124 | QLLAGLALP | 20 |
| 85 | HPIETLVDI | 18 | 92 | LRRLLLAAL | 8 | 105 | VLYPEYWKM | 20 | 115 | SLICMNTST | 20 | 125 | SAGNGSSEV | 50 |
| 98 | CNDEGLECV | 18 | 96 | LMGTVAKQL | 19 | 106 | IIRRSLPAT | 20 | 116 | VPLTSVPEL | 20 | 126 | VVSEYPSEV | 20 |

Note: Values in bold correspond to those peptides or their regions, which coincide in both predictions.

Table 2. Estimation of VEGF family receptors MHCI associated peptides in the context of HLAA.0201

| | NRP-1 NRP-2 | Secuencia Kd SEQ.ID Secuencia Kd | GLLRFVTAV 2249 167 WMYDHAKWL 5121 | VLLGAVCGV 1006 168 ILQFLIFDL 484 | WMPENIRLV 436 169 YLQVDLRFL 247 | GILSMVFYT 278 170 ALYFSRHQV 223 | LLCAVLALV 272 171 NMLGMLSGL 131 | VLLHKSLKL 134 172 WLYTLDPIL 129 | GMLGMVSGL 131 173 DIWDGIPHV 56 | FQLTGGTTV 120 174 KMEIILQFL 44 | VLATEKPTV 118 175 VLNKLHAPL 36 | GPFLFIKFV 81 176 LLGATCAGL 36 | | NRP-1 NRP-2 | Secuencia Score SEQ ID Secuencia Score | VILIGAVCGV 30 217 NMLGMLSGL 27 | GLLRFVTAV 29 218 ILQFLIFDL 26 | LLCAVLALV 28 219 DIWDGIPHV 26 | GMLGMVSGL 28 220 YLQVDLRFL 26 | ALGVILGAV 28 221 TLDPILITI 26 | VLLHKSLKL 27 222 ILAKPKMEI 25 | VLATEKPTV 26 223 VLNKLHAPL 25 | QLTGGTTVL 25 224 LLGATCAGL 25 | VILIGAVCGV 30 225 ALYFSRHQV 23 | |
|------------------------|-------------|----------------------------------|-----------------------------------|----------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------|--------------------------------|--------------------------------|-------------------------------|----------------------------|-------------|--|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|---|
| | | Kd SEQID | 1793 157 | 1055 158 | 981 159 | 929 | 161 | 369 162 | 243 163 | 177 164 | 160 165 | 159 166 | are | | Score SEQ ID | 29 207 | 27 208 | 27 209 | 26 210 | 26 211 | 212 212 | 26 213 | 25 214 | 25 215 | 300 |
| A Using BIMAS software | VEGFR-3 | Secuencia | VLLWEIFSL 1: | RLLEEKSGV 1 | VLWPDGQEV 9 | NLTDLLVNV 6 | KQAERGKWV 5 | GVIAVFFWV 3 | KLVIQNANV 2 | ALWNSAAGL 1 | TLSLSIPRV 1 | SQHDLGSYV 1 | B Using SYFPEITHI software | VEGFR-3 | Secuencia So | VLLWEIFSL | SIPGLNVTL | NLTDLLVNV | VLWPDGQEV | LLPRKSLEL | ALWNSAAGL | IMDPGEVPL | RLWLCLGLL | LIYFYVTTI | 1 1 1000 |
| Ą. | | SEQ ID | 147 | 148 | 149 | 150 | 151 | 152 | 153 | 154 | 155 | 156 | B Us | | SEQ ID | 197 | 198 | 199 | 200 | 201 | 202 | 203 | 204 | 205 | |
| | | Ρ | 1792 | 769 | 739 | 427 | 270 | 179 | 146 | 128 | 113 | 108 | | | Score | 29 | 28 | 56 | 26 | 26 | 56 | 25 | 52 | 22 | į |
| | VEGFR-2 | Secuencia | VLLWEIFSL | SLQDQGDYV | VLLAVALWL | AMFFWLLLV | VIAMFFWLL | ILLSEKNVV | LLAVALWLC | KNLDTLWKL | AVIAMFFWL | LLLVIILRT | | VEGFR-2 | Secuencia | VLLWEIFSL | LLVIILRTV | GLFCKTLTI | SIMYIVVVV | IILVGTAVI | ALMSELKIL | AASVGLPSV | SISNINVSI | AMFFWLLLV | 700000000000000000000000000000000000000 |
| | | SEQ ID | 137 | 138 | 139 | 140 | 141 | 142 | 143 | 144 | 145 | 146 | | | SEQID | 187 | 188 | 189 | 190 | 191 | 192 | 193 | 194 | 195 | |
| | | Ρ¥ | 1942 | 1792 | 901 | 257 | 182 | 179 | 160 | 137 | 118 | 112 | | | Score | 29 | 59 | 28 | 27 | 27 | 22 | 26 | 52 | 52 | L |
| | VEGFR-1 | Secuencia | FLYRDVTWI | VLLWEIFSL | KLLRGHTLV | GLLTCEATV | TLFWLLLTL | ILLSENNVV | TLNLTIMNV | CVAATLFWL | LLSIKQSNV | SLQDSGTYA | | VEGFR-1 | Secuencia | TLFWLLLTL | VLLWEIFSL | LGPGSSTL | LLCALLSCL | GLITCEATV | LLRGHTLVL | ALMTELKIL | KLLRGHTLV | TLNLTIMNV | 0.00 |
| | | SEQ | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 | 135 | 136 | | | SEQ | 117 | 178 | 179 | 180 | 181 | 182 | 183 | 184 | 185 | |

Note: Values in bold correspond to those peptides or their regions, which coincide in both predictions.

Applicants: Romero et al. Serial No.: 10/511,384 Filed: October 15, 2004

Response to Office Action Dated March 12, 2008

Page 5 of 15

On page 21 of the specification, please replace the paragraph beginning on line 9 with the following:

In the case of the extracellular domains 1 to 3 SEQ ID NO. 27 23 and SEQ ID NO: 28 24 (for domains 1 3) and SEQ ID NO: 29 and SEQ ID NO: 30 (for domain 3 alone), the primers used correspond to sequences SEQ ID NO: 9 and SEQ ID NO: 10. After digestion of the amplified fragment (943bp) SEQ ID NO: 25 and SEQ ID NO: 26 with endonucleases BamHI and EcoRI, the cDNA coding 1-3 domains of KDR was purified, and cloned in pAECΔ2 vector. Clones positive by restriction analysis were verified by sequencing of the corresponding DNA. The cDNA corresponding to KDR 1-3 was then subcloned KpnI/EcoRV in the already described pMAE5Δ5 vector (pMAE5Δ5 KDR1-3).

For the cloning of transmembrane and cytostolic regions of the receptor (SEQ ID NO: 25 and SEQ ID NO: 26) a two-step strategy was designed. For the insertion of the first segment, the primers corresponding to SEQ ID NO: 11 and SEQ ID NO: 12 were used. After the Xbal/BgIII digestion of this 747bp segment, the product was cloned in the pMAE5 vector, previously digested with the same enzymes, obtaining the plasmid PMAE5 KDR 747. This plasmid was digested BgIII/NotI in order to insert the remaining carboxi-terminal fragment of 1091bp that was amplified using the primers corresponding to sequences SEQ ID NO: 13 and SEQ ID NO: 14. Clones positive by restriction analysis were verified by DNA sequencing and denominated pMAE5 KDR C.